1: Biochemistry. 2003 Dec 30;42(51):15284-91. Related Articles, Links

Cellular uptake of Clostridium botulinum C2 toxin: membrane translocation of a fusion toxin requires unfolding of its dihydrofolate reductase domain.

Haug G, Wilde C, Leemhuis J, Meyer DK, Aktories K, Barth H.

Institut fur Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universitat Freiburg, Germany.

The Clostridium botulinum C2 toxin is the prototype of the family of binary actin-ADP-ribosylating toxins. C2 toxin is composed of two separated nonlinked proteins. The enzyme component C2I ADP-ribosylates actin in the cytosol of target cells. The binding/translocation component C2II mediates cell binding of the enzyme component and its translocation from acidic endosomes into the cytosol. After proteolytic activation, C2II forms heptameric pores in endosomal membranes, and most likely, C2I translocates through these pores into the cytosol. For this step, the cellular heat shock protein Hsp90 is essential. We analyzed the effect of methotrexate on the cellular uptake of a fusion toxin in which the enzyme dihydrofolate reductase (DHFR) was fused to the C-terminus of C2I. Here, we report that unfolding of C2I-DHFR is required for cellular uptake of the toxin via the C2IIa component. The C2I-DHFR fusion toxin catalyzed ADP-ribosylation of actin in vitro and was able to intoxicate cultured cells when applied together with C2IIa. Binding of the folate analogue methotrexate favors a stable three-dimensional structure of the dihydrofolate reductase domain. Pretreatment of C2I-DHFR with methotrexate prevented cleavage of C2I-DHFR by trypsin. In the presence of methotrexate, intoxication of cells with C2I-DHFR/C2II was inhibited. The presence of methotrexate diminished the translocation of the C2I-DHFR fusion toxin from endosomal compartments into the cytosol and the direct C2IIa-mediated translocation of C2I-DHFR across cell membranes. Methotrexate had no influence on the intoxication of cells with C2I/C2IIa and did not alter the C2IIa-mediated binding of C2I-DHFR to cells. The data indicate that methotrexate prevented unfolding of the C2I-DHFR fusion toxin, and thereby the translocation of methotrexate-bound C2I-DHFR from endosomes into the cytosol of target cells is inhibited.

PMID: 14690438 [PubMed - indexed for MEDLINE]